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博 士 学 位 论 文

大黄鱼群体遗传的微卫星分析及微卫星标记在石首鱼类系统进化研究中的应用

Study on the Population Genetics of *Pseudosciaena crocea* and Phylogenetic Perspective in Sciaenidae with Microsatellites

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摘 要

大黄鱼（*Pseudosciaena crocea*）是我国重要的海水养殖鱼类，研究其不同群体的遗传结构对促进大黄鱼资源的保护和开发具有重要意义。本文通过建立大黄鱼小片段基因组文库，应用 PCR 技术快速筛选含(CA)n 及(GA)n 微卫星序列的克隆，共得到 32 个含微卫星序列的阳性克隆，选择核心序列重复次数较多的 14 个序列登录 GenBank。应用所得微卫星序列的侧翼区设计引物，对大黄鱼样品进行扩增，得到 8 对（*PC1C4, PC4H12, PC5E11, PC7A2, PC7H4, PC8F5, PC10F10，PC10G6*）可在大黄鱼 DNA 样品中清晰扩增的微卫星引物。在大黄鱼群体中，各位点平均得到 6.52 个有效等位基因；观测杂合度（Ho）为 0.2471~0.6404（平均 0.4047)，期望杂合度（He）为 0.3883~0.9321（平均 0.7756）；各微卫星座位的多态性信息含量（PIC）为 0.3651~0.9281（平均 0.7545），除 *PC8F5* 表现为中度多态外，其它 7 个位点均表现出高度的多态性。在 8 个微卫星位点中，除 *PC7A2* 位点外，其余位点的等位基因频率均偏离 Hardy-Weinberg 平衡（P<0.05）。微卫星标记分析表明，我国海区大黄鱼群体仍保持一定的遗传变异水平，但也表现出较明显的近亲繁殖现象。

应用上述 8 个微卫星位点分析 4 个大黄鱼不同地理群体（舟山群体，宁德群体，漳浦群体，海口群体）的遗传结构。数据分析显示，大黄鱼不同地理群体的平均遗传分化系数为 0.0848，表明群体中大部分遗传变异来自群体内部；群体中存在明显的近亲交配（平均近交系数为 0.4367）；各地理群体间平均基因流为 2.6698，反映出不同地理群体间存在较频繁的基因交流。UPGMA 聚类分析结果显示，宁德群体与漳浦群体首先聚类（闽－粤东族），而后与舟山群体（岱衢族）聚类，海口群体（硇洲族）则与上述 3 个群体最后聚类。其结果基本反映了大黄鱼群体的地理分布特点，为首次应用分子遗传标记的方法证实徐恭昭等人依据形态及生态特征将我国沿海的大黄鱼种群分为 3 个不同的地理种群的研究结果。舟山群体与宁德、漳浦群体先聚类，与目前养殖现状中浙江、福建大黄鱼养殖区中苗种的较广泛流通有关。

分析上述 8 对大黄鱼微卫星标记引物在石首鱼科 5 个亚科 8 个属 10 个种中

的适用性。结果表明，大黄鱼微卫星标记引物在黄鱼亚科鱼类中有较高的适用性，其中位点 *PC1C4*，*PC4H12*，*PC5E11*，*PC8F5*，*PC10F10*，*PC10G6* 的引物可在小黄鱼（*P. polyactis*）和棘头梅童鱼（*C. lucidus*）中获得清晰扩增条带，位点 *PC7H4* 引物还可在小黄鱼中扩增，上述可扩增位点在两个种中均得到多个等位基因，表现出较高的多态性。大黄鱼微卫星标记引物在白姑鱼亚科、叫姑鱼亚科、牙　亚科及石首鱼亚科鱼类中也有较高比例的通用性，其中银牙　（*O.argenteus*）得到 6 个可扩增位点（*PC1C4,PC4H12,PC7A2,PC7H4,PC10F10,PC10G6*），鮸状黄姑鱼（*N.miichthioides*）、丁氏　（*W.tingi*）和眼斑拟石首鱼（*S.ocellatus*）各得到 5 个可扩增位点，浅色黄姑鱼（*N. chui*）和尖头黄鳍牙　（*C. aureus*）各得到 4 个可扩增位点，皮氏叫姑鱼（*J.belengerii*）得到 3 个可扩增位点（*PC7H4,PC10F10, PC10G6*）。引物 *PC5E11* 和 *PC8F5* 只在黄鱼亚科中通用，对其是否可作为鉴定黄鱼亚科的特异性标记，还应分析其在更多石首鱼类中的扩增情况。位点 *PC10F10* 和 *PC10G6* 引物在 10 个种中都得到清晰的扩增产物，是首次筛选到的可在石首鱼类中通用的微卫星标记，对其进行深入的研究有可能为石首鱼类的遗传多样性及系统进化提供重要的遗传信息。

应用可在小黄鱼中扩增的 7 对大黄鱼微卫星引物分析舟山小黄鱼群体的遗传多样性。扩增结果显示，各位点的等位基因数为 6-14 个，平均每个位点扩增出等位基因 8.29 个，各位点的 PIC 均大于 0.5，表明这些位点在小黄鱼群体显示出高度的多态性；各位点的平均期望杂合度为 0.7732，显示舟山小黄鱼群体仍然保持了较为丰富的遗传多样性。微卫星位点的等位基因频率偏离 Hardy-Weinberg 平衡，且多个位点表现为杂合度缺失，结合历史上小黄鱼曾因遭受过度捕捞造成比较严重的资源衰退，推测舟山小黄鱼群体在历史上经历过瓶颈效应，遗传漂变是造成微卫星位点等位基因频率偏离 Hardy-Weinberg 平衡的主要原因。

本文首次应用分子遗传标记对棘头梅童鱼的遗传多样性状况进行了分析。6 对大黄鱼微卫星引物在舟山棘头梅童鱼群体中总共扩增出 53 个等位基因，各位点平均等位基因数 8.83 个，平均有效等位基因数 5.31 个；各位点的 PIC 均大于 0.5，为高度多态性位点，因此这些微卫星位点可做为棘头梅童鱼群体遗传学研究的分子遗传标记；各微卫星位点的平均观测杂合度为 0.5301，平均期望杂合度值 0.7228，表明现有棘头梅童鱼群体仍具有较丰富的遗传多样性。Fis 值表明，除 *PC1C4* 位点在群体中出现了杂合过剩现象外，其它位点均表现为杂合缺失；

据此推测舟山棘头梅童鱼群体在历史上也经历过瓶颈效应。

应用可在 10 种石首鱼中通用的大黄鱼微卫星引物 *PC10F10* 和 *PC10G6-500* 本文首次应用核基因序列对石首鱼类的分子系统发生进行了探讨。对上述位点的微卫星扩增产物进行测序，Clustal X 软件比对分析微卫星侧翼序列特征，利用 MEGA4.0 软件绘制系统进化树。从各微卫星位点侧翼序列所构建的系统发生树来看，同一位点侧翼序列所构建的系统树间，NJ 树与 ME 树各分支的拓扑结构相似，而与 MP 树有较明显的区别；在不同位点间，往往体现出序列越长，黄鱼亚科鱼类的进化关系越接近传统树；所有各位点构建的系统树中，叫姑鱼亚科的 2 个种总是表现出最近的亲缘关系。但在黄姑鱼亚科和牙　亚科几个种的系统发育关系分析中，在不同位点所构建的系统树、不同构树法所生成的系统发生树间差别较大，因此，用目前的几个微卫星位点的侧翼序列还不足以清晰的阐明石首鱼类的系统发生关系。要应用微卫星的侧翼序列研究石首鱼类的系统发育，还应开发出更多的可通用的微卫星引物，得到更长的侧翼序列以更好的反映系统进化的信息。

关键词：微卫星标记；大黄鱼；群体遗传；石首鱼；通用性；系统发育

Study on the Population Genetics of *Pseudosciaena crocea* and Phylogenetic Perspective in Sciaenidae with Microsatellites

Abstract

Sciaenidae are the most commercially important fishes in China. Among all seawater cultured fishes, large yellow croaker (*Pseudosciaena crocea*) has the largest yield. However, wild caught sciaenidae fisheries have been in severe decline. And some species are in the danger of extinction without efficient fishery management. But the genetic background of these species remains still unknown. Therefore, it is necessary to understand the current situation of the natural resources of these species.

Microsatellite markers, which are characterized even distribution, high polymorphism, co-dominance and selective neutrality are widely employed in the studies concerning with variety evaluation, linkage-map construction and physical-map construction, genetic diversity of population and evolution.

Population genetics has recently gained popularity in the protection and management of sciaenidae. In this paper, the genetic diversity of *P. crocea* and some other sciaenidae were evaluated with microsatellite markers.

In the first part of this paper, a partial genomic library of *P. crocea* was constructed, and then microsatellites were isolated. The PCR-based screening library method was used to isolate microsatellite loci containing the repeat motif of (CA)n and (GA)n. A total of 55 positive clones were isolated. Among them, 32 positive clones were sequenced, of which 14 sequences were submitted to GenBank, with the accession number being EF635863 to EF635876. 8 pairs of primers were designed on the basis of the unique sequences flanking each repeat motif by using Primer3. All these primers could successfully be amplified in large yellow croakers.

In the second part of this paper, 92 individual of large yellow croakers from four stocks were applied to evaluate polymorphism of these markers and the prelimilary

genetic structure of these stocks were analyzed. GENPOP (v.1.32)was used to analyse the allele data. The number of the alleles per locus varied from 6 to 22. Mean observed heterozygosities (Ho) and expected heterozygosities (He), were 0.4047 and 0.7756, respectively. These markers showed that the polymorphism information content (PIC) were between 0.3651 and 0.9281. The observed allele frequency of most of microsatellites loci deviated Hardy-Weinberg equilibrium, the main reason for which may be that the population mating system of *P. crocea* may not follow the random mating principle.

Genetic differentiation were not significant between stocks (F_{ST}=0.0848). F_{IS} values within stocks were high (average 0.4367). The gene flow between all four stocks was 2.6998. The data showed that there were significant gene exchanges between stocks. All sampled stocks were tested and it was found that every stock contained unique alleles. The findings indicated that there were variations among the different stocks of the *P. crocea* populations, which might be caused by the large geographical differences of tested sample stocks. It implied that the germplasm of large yellow croaker could renew in some measure if suitable conservation and management strategies were adopted.

The UPGMA tree of the four stocks of *P. crocea* showed that the stocks of Ningde and Zhangpu matched firstly, we could infer that they might belong to the same geographical population of Minyuedong population. Then, they matched with Zhoushan stock which belongs to Dai-ju population. Finally, they matched with Haikou stock which might represent Naozhou population. These findings reflected the distrubtion of *P. crocea* populations and the actual conditions of culture. They were the first molecular proof that similar to the conclusion drawn by Xu gongzhao *et al*., which showed that there were three geographical populations of *P. crocea* along the coast of China sea.

Studies have indicated that there was conservation of flanking regions of microsatellite sequences among related species. If the 8 microsatellites isolated from large yellow croaker had conservative characteristics, costly and time-consuming operations would be avoided to isolate microsatellites from other sciaenidae and it

would serve as a prerequisite for inter-specific comparisons.

In the third part of this paper, the primers of 8 microsatellites loci developed from *P. crocea* were amplified in ten species of Sciaenidae fishes which were collected from the coast of Zhejiang Province and Fujian Province. We found that the ratio of successful amplifications in species belonging to the same genus or the same subfamily as the target species was high. In the subfamily of Pseudosciaeninae, 7 loci (*PC1C4*, *PC4H12*, *PC5E11*, *PC7H4*, *PC8F5*, *PC10F10*, *PC10G6*) could be amplified in *Pseudosciaena polyactis*. In *Collichthys lucidus*, there were 6 of the 8 loci, could acquire legible amplified products, except for *PC4H12* and *PC7A2*. Primers of these loci were also useful in species of Argyrosominae, Johniinae, Otolithinae and Sciaeninae. 6 loci (*PC1C4*, *PC4H12*, *PC7A2*, *PC7H4*, *PC10F10*, *PC10G6*) were amplified successfully in *Otolithes argenteus*. Loci *PC1C4*, *PC4H12*, *PC7H4*, *PC10F10*, *PC10G6* could obtain plainly discernible amplified bands in *Nibea miichthioides* and *Sciaenops ocellatus*, while *PC1C4*, *PC7A2*, *PC7H4*, *PC10F10*, *PC10G6* in *Wak tingi*. Loci *PC1C4*, *PC7A2*, *PC10F10*, *PC10G6* and loci *PC1C4*, *PC7H4*, *PC10F10*, *PC10G6* had been amplified in *Nibea chui* and *Chrysochir aureus*, respectively. Meanwhile, only 3 loci (*PC7H4*, *PC10F10*, *PC10G6*) were amplified successfully in *Johnius belengerii*. Cross-species amplification data also indicated that allelic diversity was very low in nontarget species when compared with target species in some cases. For example, locus *PC10F10* had high allelic diversity in *P. crocea* but it had only one allele in *S. ocellatus*. Loci *PC5E11* and *PC8F5* were amplified in subfamily Pseudosciaeninae only. Maybe they were specific loci in Pseudosciaeninae. This suggested that we could use these loci to identify species of Pseudosciaeninae from other sciaenid fishes. In addition, differences were found between closely related sciaenid fishes in average allelic length and the number of alleles which had been scored. Thus the microsatellite's footprints of each species might be used to distinguish closely related species, while more data were still needed to confirm the inference. Furthermore, the design of highly versatile MFR-primers might be useful for population-level work in the species belonging to this highly species-rich suborder of fishes.